CHANGES IN THE SKIN CAUSED BY TERPENES

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INTRODUCTION

Terpenes are used in pharmaceutical and cosmetic skin products, and are generally regarded as a safe. Nevertheless, they cause noticeable changes in the *stratum corneum* structure and increase penetration of active molecules into deeper skin layers or even through the skin. There are several *in vitro* and *in vivo* techniques, which are used to visualize and describe the changes in human skin after the application of xenobiotics.

The aim of the study was to use fluorescence microscopy (FM), atomic force microscopy (AFM) and scanning electron microscopy (SEM) to visualise structural changes in *stratum corneum* and to carry out *in vitro* procedure to assess the viability of the skin epidermis EpiDerm® model after the application of terpenes. The following terpenes incorporated into dermatological vehicles were tested: racemic menthol, levomenthol, eucalyptol and camphor.

EXPERIMENTAL METHODS

In vitro skin irritation test

In vitro test was performed according to *in vitro* experiment S.O.P ECVAM: *In vitro* skin irritation test: human skin model, EpiDerm®-200 [1].

Selected formulations with terpenes and pure grape seed oil, ethanol and carbomer gel as the reference samples were applied on the tissue. The effects were shown as changes in the viability of cells.

Ex vivo application of terpenes and FM

Samples of separated *epidermis* with *stratum corneum* obtained from human cadaver skin were used. Grape seed oil, ethanol and carbomer gel with different terpenes and their concentrations were applied.

Following the application of the formulations and the incubation of the skin samples, fluorescent dyes were put onto the skin surface to visualize corneocytes by FM. Samples were observed using 10, 40 and 100x magnifications.

In vivo application of terpenes and AFM and SEM

For AFM and SEM microscopic examinations, tape stripping method was used [2]. After *in vivo* application of selected formulations on the inner part of forearms, tape strips were pressed homogenously on the selected area of the skin and the *stratum corneum* layer was collected. Isolated corneocytes were observed and analysed with AFM and SEM.

RESULTS AND DISCUSSION

In vitro irritation test demonstrated the way in which terpenes affect the life layers of *epidermis*. After the application of selected formulations, the differences in the viability of EpiDerm® tissue were observed and depicted in Fig. 1.

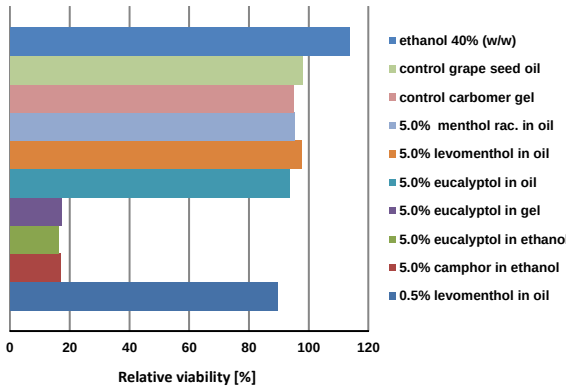

Figure 1: Viability of EpiDerm® after the application of selected formulations.

The irritation potential of tested materials according to an EU classification is predicted if the mean relative tissue viability of three individual tissues exposed to the test substance is reduced below 50% of the mean viability of the negative controls.

The results show that 5% camphor solution in 40% ethanol, 5% eucalyptol solution in ethanol and 5% eucalyptol in gel have the most destructive potential on EpiDerm® tissue.

Stratum corneum shows autofluorescence but in order to enhance the intensity of fluorescence and to facilitate the distinction between corneocytes and intercellular lipids, fluorescent dyes with different lipophilicity were used. The highest intensity of fluorescence as well as structural changes in the *stratum corneum* was observed when camphor in ethanol was applied. 3D images were constructed to show the differences in the intensity of fluorescence (Fig. 2 and 3).

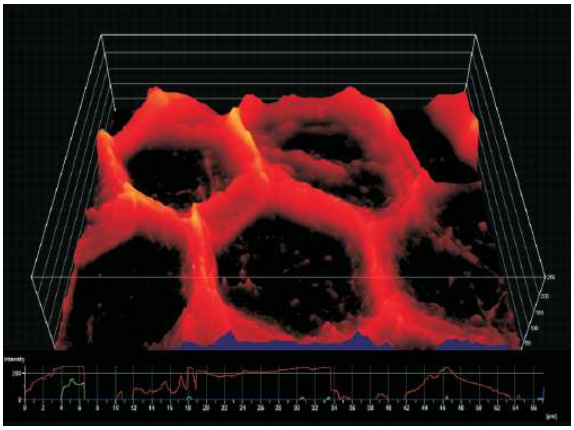

Figure 2: Fluorescence intensity of *stratum corneum* treated with ethanol (control) and lipophilic rhodamine B hexyl ester dye.

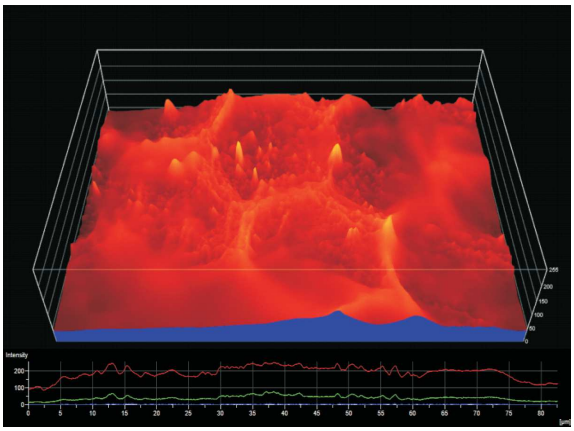

Figure 3: Fluorescence intensity of *stratum corneum* treated with 5% camphor in ethanol and lipophilic rhodamine B hexyl ester dye.

The results have shown that terpenes dissolved in ethanol and applied *ex vivo* have the most destructive effects on *stratum corneum*, while dissolved in grape seed oil – the smallest. In addition, the impact of the concentration of terpenes in the vehicles on the strength of their action on *stratum corneum*, such as the arrangement of corneocytes, was shown as a difference in the disposition of fluorophores and in the intensity of fluorescence.

AFM images can demonstrate the changes in the topography of individual corneocytes after the application of the formulations. At the same time, AFM provides unique information with a high resolution, like surface roughness. Typical 3D AFM topography of corneocytes is presented in Fig. 4. SEM technique allows to present single cell and space between them, but, as with AFM, no significant changes after treatment with terpenes were observed.

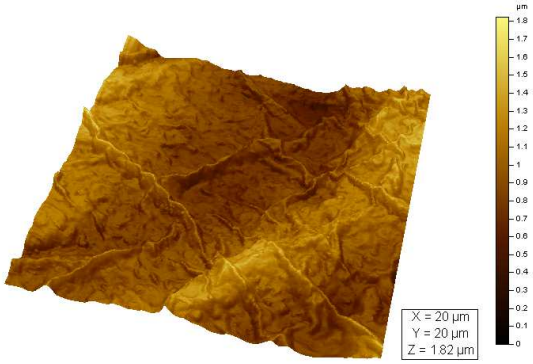

Figure 4: 3D topography of corneocytes.

CONCLUSIONS

Dermal irritation *in vitro* test has shown a highly destructive impact of eucalyptol and camphor on the life layers of *epidermis*. It was confirmed by FM after *ex vivo* application of terpenes. However, after *in vivo* application, AFM and SEM examination of the treated and untreated probes has shown no significant changes in *stratum corneum* and individual corneocytes.

ACKNOWLEDGEMENTS

This study was supported by grant No N N405 303536 from the Ministry of Science and Higher Education (Poland).

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